Review

Nanoparticle-Based Targeted Therapeutics in Head-And-Neck Cancer

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Abstract

Head-and-neck cancer is a major form of the disease worldwide. Treatment consists of surgery, radiation therapy and chemotherapy, but these have not resulted in improved survival rates over the past few decades. Versatile nanoparticles, with selective tumor targeting, are considered to have the potential to improve these poor outcomes. Application of nanoparticle-based targeted therapeutics has extended into many areas, including gene silencing, chemotherapeutic drug delivery, radiosensitization, photothermal therapy, and has shown much promise. In this review, we discuss recent advances in the field of nanoparticle-mediated targeted therapeutics for head-and-neck cancer, with an emphasis on the description of targeting points, including future perspectives.

Key words: Nanoparticles, targeted therapeutics, head-and-neck cancer, RNA interference, drug delivery, radiosensitization, photothermal therapy

Introduction

Head-and-neck cancer is the sixth most common cancer worldwide, with an estimated 900,000 new cases and 350,000 mortalities per year, accounting for 5–6% of all cancer cases, and affecting males more than twice as often as females [1, 2]. Almost all of these cancers are squamous cell carcinomas of the head-and-neck (HNSCC), which arise in the paranasal sinuses, nasal cavity, oral cavity, pharynx, and larynx.

Tobacco and alcohol consumption are widely accepted as the most significant risk factors for HNSCC [3, 4]. However, infection with human papillomavirus (HPV), particularly HPV types 16 and 18, has been associated with an increase in oropharyngeal cancer in younger nonsmokers [5].

Despite recent advances in the diagnosis and treatments for patients with HNSCC, the overall outcomes and treatment-associated toxicities remain disappointing [6]. One half of newly diagnosed cases are in the advanced stages (3 or 4), leading to high death rates. The average 5-year survival rate for all stages, based on end-result data is ~60%. However, 50–60% of local HNSCC patients will progress to regional or distant relapses within 2 years, with a decrease in survival rate from 80% down to 50 or 35% [7]. Recurrent/metastatic patients have a median survival of less than 1 year [8].

HNSCC is a deadly and disfiguring disease, and treatment of tumors is complicated and always requires a multidisciplinary approach [9]. Surgical resection and/or radiotherapy have long been regarded as the standard treatment for HNSCC, especially in the early stages, while chemotherapy can be added as an adjunct. However, because of the complex anatomy and vital function of the facial structures, the extent of surgery will always be limited. Conventional treatments are far from perfect, either having low efficacy or resulting in severe side effects [10]. Recent researches have focused on advanced chemotherapy or radiotherapy to preserve organ function and improve the quality of life [6, 9].

Nonspecific distribution is an important factor contributing to the side effects and poor clinical outcomes in conventional treatments. Targeted therapeutics, aimed at diseased tissue, have emerged as promising alternatives over conventional approaches, and overcome certain drawbacks, such as nonspecific distribution and tumor resistance. Specific antitumor effects can be achieved by blocking gene expression vital for tumorigenicity and/or tumor growth, or guiding coupled drug molecules into tumor cells in combination with an over-expressed receptor.

Nanoparticles (NPs), a class of versatile materials with diameters of 1–100 nm, can act as carriers for many drugs, imaging agents, and targeting ligands. Various types of NP have been developed as carriers for therapeutic agents, and the NP-based targeted delivery attracts more attention, which contains passive targeting and active targeting. Nano-sized particles tend to accumulate in tumor tissues, without conjugating to any tumor-specific targeting moiety; this is known as the enhanced permeability and retention (EPR) effect, which realize the passive targeting [11]. This therapeutic effect is achieved because of the abundant but leaky vasculature and impaired lymphatic drainage in tumor tissues, arising as a result of superfast growth and insufficient nutrient supply [12, 13]. Nanoparticles, because of their small size, can extravasate through endothelium or penetrate microcapillaries of the tumors. Beyond the passive EPR effect, nanoparticles provide a surface for the attachment of specific molecular motifs to enable facilitated internalization and active tumor targeting. Indeed, nano-based active targeting has gradually attracted the focus from passive targeting systems.

Recent research has highlighted many advantages of a targeted nanomedicine approach in a combined therapy for treating HNSCC, such as enhanced preferential tumor-killing efficiency and reduced toxicity to healthy tissues [14]. This review article outlines nanoparticle-mediated targeted therapeutics for head-and-neck cancer, with the aim of identifying new approaches to improve the prognosis of patients with HNSCC. The PubMed, Web of Science, and Google Scholar databases were used.

Characteristics and advantages of nanoparticle carriers

Size and size distribution are the most important characteristics of nanoparticles, determining the endosomatic distribution, biological fate, toxicity, and targeting ability [15]. Small size facilitates relatively high cell uptake. Small particles have a larger surface area-to-volume ratio, exposing more attached drugs near the surface, thus leading to a faster drug release. Larger particles have larger cores, encapsulating more

drugs inside and presenting a slower release rate. So, tuning of particle size provides a means of controlling drug release rate [16].

Nanoparticles with non-modified surface can be recognized by the host immune system once in the blood stream, and massively cleared from the circulation by mononuclear phagocyte system (MPS) such as liver, spleen, lungs and bone marrow, which significantly shortens the circulation time and leads to targeting failure [17]. Novel nanoparticles coating with hydrophilic polymers/surfactants or formulating with biodegradable copolymers with hydrophilic characteristics, e.g., polyethylene glycol (PEG), can evade the human immune system [18]. PEG molecules with brush-like and intermediate configurations prevents opsonization and reduces phagocytosis [19].

The zeta potential is another useful character to describe the surface charge property and determine whether the charged load should be encapsulated within the center or on the surface of the nanoparticle. Surface zeta potential above ± 30 mV prevents aggregation of the particles and stabilizes nanoparticles in suspension [20].

A successful nanodelivery system should possess a high drug-loading capacity and exhibit a controlled drug release rate, which can be modified by drug-polymer interactions, the molecular weight, solubility, diffusion, biodegradation, end functional groups in either the drug or matrix [21-23].

With the development of nanotechnology, various types of NP have been applied in medical field to carry therapeutic agents. Liposomes are one of the most widely used carriers, acting as "contact-facilitated drug delivery", which displays as when binding or interacting with the targeted cell membrane, the lipid-lipid exchange with the lipid monolayer of the nanoparticle enhances, thus accelerateing the convective flux of lipophilic drugs (e.g., paclitaxel) to dissolve through the outer lipid membrane of the nanoparticles to targeted cells membrane [24]. Block-copolymer micelles (e.g. poly(amino acid)) are amphiphilic nanospheres assemblized with a hydrophobic core available for accommodating lipophilic drugs and a hydrophilic brush-like coronal shell to make the micelle water soluble and prolong their circulation time, thereby suitable for delivery of the poorly soluble contents [25].

Degradable polymersomes are hollow shell nanoparticles with thick membranes comprised of two layers of synthetic polymers and an aqueous lumen, tending to break down in the acidic environment and release drugs within tumor cell endosomes, which is called pH-triggered release [26]. Polymersomes have been used to encapsulate paclitaxel and doxorubicin for passive delivery into cancer cells. Paclitaxel, which

is water insoluble, embeds within the shell of polymersomes. While doxorubicin, which is water-soluble, stays within the interior lumen of the polymersome until it degrades. The combination of polymersome and drug spontaneously self-assembles when mixed together. The cocktails of paclitaxel and doxorubicin lead to significant tumor regression [27].

Single-walled carbon nano-tubes (SWCNTs) are synthesized by covalently attaching multiple copies of tumor-specific monoclonal antibodies, radiation ion chelates and fluorescent probes [28], overcoming the limitation of impeded targeting ability resulted from too much chemical bonds interacted between antibody and drug molecules, showing great potential to carry multiple drug payloads. Multidrug resistance (MDR) of tumor cells developed through a variety of molecular mechanisms is a serious problem in chemotherapy. Attacking tumors with more than one kind of drugs at a time can reduce the possibility of their escaping from treatment and overcome MDR [29].

.Programmed drug delivery, named from the ability to alter the structure and properties of nanocarriers when delivering, can be achieved by incorporating of molecular sensors that respond to physical or biological stimuli, including changes in pH, redox, potential, or enzymes [30]. Nanomaterials have emerged to create a promising drug-delivery system with advantages like enhanced stability, ease of surface modification, surface for targeted delivery, improved bioavailability, sustained drug release and assistant to solubilize drugs for systemic delivery.

1. Antisense oligonucleotides (ASOs)

The activity of oncogenes—including, myc, fos, ras and certain viruses such as HSV-1 and HPV-is important in tumorigenicity. Aberrant activation of oncogenes evokes a complex network of signaling pathways that interfere with biological systems, and blocking any of the conducting molecules in a relevant network may inhibit tumor growth.

Antisense oligonucleotides (ASOs) are an appealing gene-silencing strategy; they are induced by a single strand of oligonucleotides, targeted at the complementary region of the oncogene mRNA by Watson–Crick base pairing, and can downregulate oncogene expression and abrogate tumor growth [31]. The mechanism is associated with the activation of endogenous ribonuclease H and subsequent exonuclease cleavage of the associated mRNA [32].

The major limitation of ASO-mediated gene silencing therapy is the difficulty in delivering a sufficient quantity of antisense molecules into tumor cells. Introducing a phosphorothioate backbone improves the stability of the ASOs, but there are accompanying drawbacks, such as increased toxicity and diminished

affinity for the target sequence [33]. The 2'-methoxyethyl modified second-generation phosphorothioate ASOs have shown higher efficacy in cancer gene therapy, and are at present undergoing clinical trials [34].

Carriers for ASOs can be categorized into two groups; natural (viruses and bacteria) and nonviral. Viral vectors provide efficient delivery but also have several drawbacks, such as insertional mutagenesis and immunogenicity [35]. In contrast, nonviral carriers are safer and easier to produce. Cationic liposomes (e.g. Lipofectamine® 2000) have been trialed and are some of the most widely used nonviral nanocarriers for ASO delivery; they have low toxicity, are non-immunogenic, only slightly inflammatory, and are easier to obtain than viral vectors, while their chief disadvantages consist of relatively low transfection efficiency and/or a shortened effective gene silencing time [36]. Cationic liposomes increase nucleic acid uptake into cells compared to standard liposomes, with minimal toxicity [37].

1.1 Glucose transporter-1 (Glut-1)

Malignant cells exhibit increased glucose consumption and lactate production, even under normal oxygen conditions, known as the Warburg effect, or aerobic glycolysis [38, 39]. The Warburg effect has received increased attention lately, especially following the rapid development of FDG-PET (FDG, fluorodeoxyglucose) for tumor imaging [40]. The glucose transporter-1 (Glut-1) is a membrane protein that facilitates the intracellular uptake of glucose. Elevated expression of Glut-1 has been observed in several cancer types and has been identified as a valuable prognostic indicator [41-43]. In several experiments, Glut-1 expression was correlated with lymph node metastasis, poor survival, and clinical stage HNSCC, and increased Glut-1 expression can be an independent predictor of survival in laryngeal carcinoma [44, 45].

Recently, the crystal structure of human Glut-1 was identified as in an inward-open conformation. This major breakthrough serves as a basis for understanding the functional mechanism of Glut-1 and for the development of potential targeted therapeutic agents [46].

A pcDNA3.1(+) eukaryotic expression system vector containing the antisense Glut-1 gene was constructed, followed by successful transfection into Hep-2 laryngeal carcinoma cells [47]. Another study investigated the biological effects of plasmid-derived antisense RNA against the Glut-l gene in Hep-2 cells, and reported inhibited proliferation and decreased glucose uptake [40].

1.2 Epidermal growth factor receptor (EGFR) and Stat3

He *et al.* investigated the intratumoral transfer of cationic liposome-mediated antisense EGFR plasmids into HNSCC subcutaneous xenografts, which resulted in suppression of EGFR protein expression, increased tumor cells apoptosis, and inhibition of tumor growth [48]. Grandis *et al.* aimed to demonstrate that EGFR-mediated Stat3 activation contributed to the uncontrolled acceleration of tumor growth by an anti-apoptosis mechanism, and found that inhibition of Stat3 activation via a liposome-mediated Stat3 antisense plasmid resulted in inhibited tumor growth and stimulated apoptosis in HNSCC xenograft models [49, 50].

Intratumoral administration of antisense oligonucleotides showed an antitumor effect in xenograft models of squamous cell carcinoma of the head-and-neck (SCCHN), but limited clinical application. Studies underway at present aim to improve systemic administration [51].

1.3 CK2 and NF-κB

Protein kinase CK2 consists of two catalytic subunits (42 kDa α, 38 kDa α') and a regulatory subunit (28 kDa β), and forms holoenzyme tetramers such as α2β2, αα'β2,or α'2β2 [52]. CK2 plays a key role in many diseases, including prostate, breast, kidney, and lung cancers, via modulation of cell proliferation and differentiation and anti-apoptosis mechanisms [53, 54]. CK2 is elevated in HNSCC, and is associated with aggressive tumor behavior and a poor prognosis, indicating that it may be an effective therapeutic target [53, 55]. Brown *et al.* reported the antitumor effects of anti-CK2α/α' oligodeoxynucleotide (ODN) encapsulated in sub-50-nm tenfibgen-based nanocapsules in HNSCC xenograft models, which was accompanied by suppression of NF-κB and modulation of the expression of key genes [56].

1.4 Transforming growth factor alpha (TGF-α)

TGF-α is a polypeptide that interacts with EGFR [57]. Over-expression of TGF-α alone, or highly co-expressed TGF-α and EGFR, has been implicated in the oncogenesis of many cancers, including HNSCC, and is an independent prognostic factor for the survival of patients with primary HNSCC [58, 59]. *In vitro* studies have shown that downregulation of TGF-α expression via ASOs successfully inhibited proliferation of HNSCC [60]. Endo *et al.* examined the antitumor effects of cationic liposome-mediated antisense human TGF-α in a HNSCC xenograft model, and reported positive results [61].

1.5 Survivin

Survivin is a member of the inhibition of apoptosis (IAP) gene family, prominently expressed in all common human cancers, but barely expressed in normal adult tissues [62]. Survivin has multiple functions, including inhibition of apoptosis and cell-cycle regulation, and plays a key role in carcinogenesis and tumor progression [63]. Overexpression of survivin occurs in HNSCC and is regarded as a prognostic marker [64, 65]. Xiang *et al.* transformed survivin-ASOs via liposomes into the Hep2 human laryngeal carcinoma cell line, which resulted in significant downregulation of survivin gene expression and protein levels, and enhanced antitumor effects *in vitro* and *in vivo* [66].

2. RNA interference (RNAi)

Investigation into the use of RNAi, another attractive possibility for cancer gene therapies, has been conducted increasingly since the award of the Nobel Prize in 1998 [67]. RNAi is defined as post-transcriptional gene silencing, initiated by approximately 21- or 22-nucleotide double-stranded RNAs (dsRNA) with a sequence homologous to that of the targeted gene [68]. The small interfering RNA (siRNA) incorporates into the RNA-induced silencing complex (RISC) upon reaching the cytoplasm, where the duplex is separated and one strand guides the RISC to combine with the targeted mRNA, bearing an exact complementary sequence. This perfect match results in degradation or translation blockage of mRNA, thus inhibiting expression of the relevant gene [69-71].

siRNA and ASOs function by inducing degradation of targeted messenger RNA. The gene silencing efficacy of duplex siRNAs and single-stranded antisense RNA is at present undergoing evaluation. Bertrand *et al.* compared the efficacies of antisense RNA and siRNA delivered by Grassy Stunt Virus (GSV) in a HeLa cell culture and in xenografted mice; siRNA was more effective, possibly due to enhanced resistance to nuclease degradation due to the formation of the RISC [72]. Xu *et al.* found that double-stranded siRNAs exhibited higher gene silencing efficacy than ASOs when targeted to multiple sites of exogenous luciferase mRNA and endogenous CD46 mRNA in mammalian cells [73]. However, Holen *et al.* reported that ASOs were less effective in reducing targeted mRNA (Human Tissue Factor) expression, but reached a peak faster than the duplex siRNA in HaCaT cells. As an excess of inactive double-stranded siRNA competed in a sequence-independent manner with ASOs, it was concluded both gene silencing strategies shared a pathway [74].

RNAi-based technology has shown great potential in targeted cancer therapy by suppressing the expression of genes associated with tumor growth [75]. Other molecular targets with a high specificity for HNSCCs have also been investigated, such as epidermal growth factor receptor (EGFR) and folic acid. The interference activity of siRNA occurs primarily in the cytoplasm. However, siRNAs cannot readily pass through the cell membrane due to their high molecular weight and negative charge. Furthermore, after systemic administration, nonspecific distribution of siRNA decreases local concentrations, serum RNase rapidly hydrolyzes naked siRNA, and rapid renal excretion and unexpected reticuloendothelial uptake further reduce its effective duration [76, 77]. Therefore, a multi-functional delivery system that protects and introduces siRNA into targeted cells is important for successful gene knockdown. Nanotechnology has been applied to assist siRNA delivery and has increased its stability and facilitated its introduction into malignant cancer cells [78, 79].

2.1 Epidermal growth factor receptor (EGFR)

EGFR, a member of the ErbB receptor family (Her-1, Her-2, Her-3, and Her-4), is composed of an extracellular ligand-binding domain, a hydrophobic transmembrane segment, and an intracellular tyrosine kinase (TK) domain. The extracellular domain provides a binding site for the endogenous ligand, epidermal growth factor (EGF) or TGF-α, and the binding interaction induces subsequent receptor-mediated internalization and auto-activation of intracellular tyrosine kinase (TK), which is closely related to other vital intracellular signaling pathways [80].

Over-expression of EGFR is detected in over 90% of HNSCC cases and is associated with a poor treatment response and a worse prognosis [80, 81]. An increase in EGFR has been implicated in oncogenicity through activation of a series of aberrant downstream cell proliferation signaling pathways, differentiation, anti-apoptosis, and invasiveness [82]. Hence, inhibiting the function of EGFR to interrupt the mechanisms of tumor growth by siRNA technology has attracted much attention. Cho *et al.* investigated a polyelectrolyte nanocomplex composed of PLR and DEX for the delivery of EGFR-siRNA in an HNSCC model. Results showed an increased efficiency in EGFR-siRNA cell uptake and EGFR gene silencing in Hep-2 and FaDu cells, and efficient tumor growth inhibition *in vivo* [83].

2.2 Ribonucleotide reductase M2 (RRM2)

RRM2, is the M2 subunit of ribonucleotide reductase (RR), and expression is increased 3–7-fold when cell the cycle passes from the G1- to S-phase; it plays a critical role in DNA synthesis by modulating the enzymatic activity of RR in the conversion of ribonucleotide 5'-diphosphates to 2'-deoxyribonucleotides [84, 85]. Overexpression of RRM2 and the subsequent elevated RR activity are associated with tumorigenesis and tumor progression, suggesting that RRM2 could be a potential target for tumor diagnosis and therapy. Recent research has indicated some success in using RRM2-siRNA against a wide range of tumors, including non-small-cell lung cancer (NSCLC), pancreatic adenocarcinoma, bladder cancer, leukemia, and some solid tumors [86].

CALAA-01 is a nano-sized siRNA therapeutic that contains: (i) a liner, cyclodextrin-based polymer (CDP), (ii) human transferrin protein (hTf) ligands displayed on the surface as the targeting moiety to engage transferrin receptors (TfR), (iii) a hydrophilic polymer used to stabilize nanoparticles in biological fluids, and (iv) siRNA targeting to RRM2 [87]. TfR is upregulated in malignant cells, and use of the hTf moiety in the delivery system helps to achieve more specific and efficient delivery of siRNA [88, 89]. The CALAA-01 delivery system has had positive anti-tumor results and has been shown to be safe in many cancer models. The first siRNA clinical trial is underway (clinical trial registration number, NCT00689065) to test the effectiveness of CALAA-01 in systemic delivery of siRNA in patients with solid cancers [87].

Rahman *et al.* successfully delivered RRM2-siRNA via CALAA-01 nanoparticles to suppress HNSCC tumor growth both *in vitro* and *in vivo*, without any signs of adverse effects or toxicity [90]. The underlying mechanisms were also investigated, and degradation of Bcl-2 was identified as the key determinant in tumor cell apoptosis [91].

2.3 Human rhomboid family 1 gene (RHBDF1)

The rhomboid family of genes is highly conserved, encoding a group of seven transmembrane proteins which function in diverse processes, including protein cleavage, signaling pathway modulation [92, 93], apoptosis [94], mitochondrial membrane fusion [95], endoplasmic reticulum-associated degradation [96], and others. Recent research has highlighted a connection between the RHBDF1 gene and a variety of human diseases, such as leukemia [97] and breast cancer [98], and research into gene silencing therapies is ongoing [99]. The RHBDF1 gene is the first member of the rhomboid family. It is located on the endoplasmic reticulum and the Golgi apparatus, and interacts with TGF-α ligands, which is followed by EGFR activation [100].

Yan *et al.* demonstrated that elevated expression of RHBDF1 was essential in epithelial cancer cell

growth. Silencing the RHBDF1 gene with siRNAs resulted in apoptosis in breast cancer MDA-MB-435 cells, and autophagy in HNSCC1483 cells, probably caused by the downregulation of activated AKT and other extracellular growth signals. Additionally, systemic administration using a histidine-lysine polymer (HKP) nanoparticle delivery system increased the deposit of RHBDF1-siRNA in MDA-MB-435 and 1483 xenograft tumors, which led to marked gene silencing and inhibition of tumor growth, compared to nude siRNA [101]. Zou *et al.* found that RHBDF1 participated in G protein-coupled receptor (GPCR)-mediated transactivation of latent EGFR ligands in HNSCCs [102].

3. Immunotherapy

Epidermal growth factor receptor (EGFR)

There are two complementary strategies in EGFR immunotherapy; monoclonal antibodies (e.g., Cetuximab; CET) and small molecular TK inhibitors (e.g., Erlotinib). CET (Erbitux), a chimeric (mouse/human) IgG monoclonal antibody that binds exclusively to EGFR and blocks its function through competitive inhibition, has been approved by the U.S. Food & Drug Administration (FDA) for treatment of colorectal and lung cancers, and HNSCC. TK inhibitors target the intracellular domain of EGFR, competing with adenosine triphosphate (ATP) for binding sites. Though immunotherapy alone has exhibited a limited effect in clinical practice in treating HNSCC, a combination with other therapeutic methods has yielded some positive results [103] [104].

Albumin is an essential carrier for binding and transporting various functional molecules throughout the circulatory system. Recently, nanosized formations of albumin have shown promise as a drug-delivery system. The glutaraldehyde cross-linked albumin nanoparticle is ~100 nm in diameter. Altintas *et al.* modified the surface of the albumin nanoparticles with bifunctional PEG 3500 and a nanobody (EGa1) against EGFR, which showed 40-fold greater affinity to EGFR-positive HNSCC 14C cell lines compared to PEGylated cells. The EGa1-PEG functionalized nanoparticles were used to deliver multikinase inhibitor 17864-Lx, a platinum-bound sunitinib analogue, which resulted in a constant rate of drug release and an antiproliferative effect on 14C cells [105].

4. DRUG DELIVERY

Chemotherapy is generally used alongside surgery and/or radiotherapy in advanced cancer cases. The most common chemotherapeutic agents used are platinum-based drugs (cisplatin or carboplatin) and combinations with taxanes (e.g., docetaxel) or 5-fluorouracil. However, conventional delivery methods of chemotherapeutic agents have several limitations: Firstly, some drugs have poor solubility and low bioavailability and contain toxic solvents in their formulation. Secondly, they have a short circulation time because of their physiological instability, degradation, and clearance. Thirdly, the nonspecific distribution of the drugs limits the concentration achieved in the tumor, and causes harmful side-effects because of their unwanted accumulation in healthy tissues. Clinical studies revealed that approximately 30% of patients with advanced HNSCC responded to a single agent, such as cisplatin or 5-FU, but no improvement in overall survival was observed [106]. A combination of chemotherapeutic agents did improve the drug response but had no effect on overall survival [107]. Therefore, advanced drug delivery systems (DDS), based on nanotechnology and a tumor-targeted strategy, hold considerable potential to enhance chemotherapeutic efficacy.

Passive targeting

Passive diffusion is the major internalization mechanism of free drugs into tumor cells, which tends to activate the efflux pump and removes drugs from the cells. Fortunately, nano-sized complexes can be internalized by tumor cells via endocytosis, thus avoiding the increased efflux pump mechanism and reducing drug resistance [29]. Zhou *et al.* developed a series of amphiphilic chitosan derivatives by grafting deoxycholic acid and hydrophilic molecules, with sizes of 160–240 nm, for loading of the hydrophobic drugs, paclitaxel (PTX) and doxorubicin (DOX). These nanoparticles showed sustained and controlled drug release, the rate of which could be adjusted by changing the degree of substitution (DS) of deoxycholic acid and hydrophilic molecules, and the pH of the release medium [108]. Several nanoparticle formulations of chemotherapeutic agents have been approved for clinical use; Abraxane is a nanoparticle formulation of albumin and paclitaxel, and Genexol-PM is a polymeric nanoparticle formulation of paclitaxel.

Active targeting

As described previously, nanoparticles provide suitable attachment sites for targeting moieties. By interacting with overexpressed receptors or via specific expression on the surface of targeted cancer cells, functionalized nanosystems could further improve the efficacy and specificity of drug delivery systems. Research is concentrated on identifying more specific receptors and effective nanosystems (Table 1).

Table 1. An overview of targeted nanodevices for drug delivery in head and neck cancer management

Targeting antigen	Targeting moiety antibody	Nano-platform	Drug	Ref.
Folate receptor	folic acid	heparin-folic acid-paclitaxel (HFT)	paclitaxel	[113]
	folic acid	acetylated generation 5 dendrimers	methotrexate	[114]
	folic acid	magnetic nanoparticles	cisplatin	[115]
	folic acid	Albumin	paclitaxel	[118]
	folic acid	Liposome (plus antisense HER-2)	cisplatin, taxotere, doxorubicin, 5-fluorouracil	[133]
EGFR	EGF	SWNTs	cisplatin	[119]
	Cetuximab	carbon nanoparticle (ps: noncovalent assembly)	paclitaxel	[103] [104]
Mcl1	Mcl1 siRNA	lipid nanoparticles	mitoxantrone	[123]
	Mcl1 siRNA	lipid nanoparticles	paclitaxel	[124]
	Mcl1 siRNA	lipid nanoparticles	SAHA	[125]
CK 2	CK 2 siRNA	liposome	cisplatin	[56]
Survivin	Survivin siRNA	liposome (plus iNOS-specific inhibitor 1400W)	paclitaxel	[134]

Abbreviations: HFT, heparin-folic acid-paclitaxel; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor receptor; HER-2, human epidermal-growth-factor receptor 2; SWNTs, single-walled carbon nanotubes; Mcl1, myeloid cell leukemia sequence 1; SAHA, suberoylanilide hydroxamic acid; iNOS, inducible NO synthase

4.1 Folate receptor

Folate receptors (FRs) are glycosylphosphatidylinositol-anchored cell surface receptors with a high affinity for folic acid (FA). Though FRs are present throughout the body and have important physiological functions, they are highly expressed in a wide range of malignant cancers, such as breast, ovarian, lung, kidney and HNSCC [109]. FA is a water-soluble B vitamin, critical for DNA synthesis, and has potential as a ligand for targeted drug delivery, in terms of its low molecular weight (441 Da), stability, non-immunogenicity, and ease of synthesis. FA retains its ability to bind with FRs after conjugation with other structures, and can then be transported into cells through the FR-mediated endocytosis pathway [110]. FRs are one of the most widely investigated receptors for the targeting of drug delivery systems to FR-positive tumors [111]. Overexpression of FRs occurs in approximately half of primary HNSCCs and correlates with a worse clinical outcome, indicating a promising role for FR in targeted delivery in HNSCC patients [112].

Wang *et al.* synthesized a ternary conjugate heparin-folic acid-paclitaxel (HFT), loaded with additional paclitaxel (T), to improve the antitumor efficacy and specificity of paclitaxel for the FR-positive HNSCC KB-3-1 cell line *in vitro* and *in vivo*. The resulting nanoparticle, HFT-T, selectively recognized FR-positive cancer cells and markedly inhibited tumor growth compared to the free form of paclitaxel, without showing a resurgence of tumor growth after several weeks' treatment [113]. Ward *et al.* conjugated methotrexate and folic acid to acetylated generation 5 dendrimers, which significantly increased its chemotherapeutic performance in HNCSS *in vitro* and *in vivo* [114]. This is the first instance of dendrimers being used as a platform for loading targeting moieties and therapeutic drugs. Xie *et al.* developed a folate-conjugated cisplatin-loaded magnetic nanomedicine (CDDP-FA-ASA-MNP), which provided an alternative platform for drug delivery in HNSCC patients [115].

Dosio *et al.* conjugated paclitaxel to human serum albumin (HSA), which increased the performance of paclitaxel in three tumor cell lines, with slower elimination, continuous drug release, high cytotoxicity, and reduced systemic toxicity compared to the free drugs [116]. Further research showed that surface modification by covalent linkage of polyethylene glycol (mPEG) provided a shield, and further reduced clearance and organ uptake [117]. Folic acid was added as a targeting ligand based on previous paclitaxel–albumin mPEG derivatives. The resulting folate-mediated paclitaxel-loaded albumin complexes demonstrated increased selectivity and anti-tumor efficacy in the human nasopharyngeal epidermal carcinoma KB cell line [118].

4.2 Epidermal growth factor receptor (EGFR)

As described in the section on siRNA delivery above, EGFR can also provide a target for chemotherapeutic drug delivery. Ashwin *et al.* demonstrated that EGF-directed single-walled carbon nanotubes (SWNTs), as a delivery system for cisplatin chemotherapy, resulted in more specific and rapid drug internalization into HNSCC cells, and distinct tumor growth regression compared to a non-targeted SWNT-cisplatin control. Knockdown of the EGFR gene by siRNA blocked the accelerated drug uptake, which confirmed the importance of the EGF–EGFR interaction in this delivery system [119]. Carbon nanotubes are considered to be suitable transporters

for drug delivery, having a unique size, shape and physical properties [120].

Berlin *et al.* reported another EGFR-targeted carbon nanoparticle for drug delivery. All three if its components, a PFG-functionalized carbon nanovector, CET and paclitaxel, were assembled through noncovalent interactions by simple physisorption, which is essential in creating personalized medicines [103]. A previous report revealed the equivalent cytotoxicity of noncovalent paclitaxel-loaded carbon nanoparticles when added to a commercial formulation of paclitaxel (PTX/Cremophor, solution of PTX in Cremophor) [121]. The addition of CET, a monoclonal antibody targeted to EGFR, facilitated the specific uptake of paclitaxel by EGFR+ cells (OSC-19) *in vitro* [103]. In a FaDu -and OSC-19- cell-derived orthotopic model of tongue cancer, this novel targeting delivery system showed marked anti-tumor activity but disappointingly, no significant difference compared to the other PTX treatment groups was observed [104].

4.3 Myeloid cell leukemia sequence 1 (Mcl1)

Mcl1, belongs to the Bcl-2 family of apoptosis-regulating proteins, and exerts a negative effect on apoptosis-induction resulting from chemotherapeutic agents [122]. Cationic lipid nanoparticle-based Mcl1-siRNA loading with mitoxantrone showed enhanced antitumor activity compared to Lipofectamine® 2000-mediated transfection of siMcl-1 [123]. Yu *et al.* loaded cationic lipid nanoparticles with PTX and Mcl1-siRNA and observed the highest cellular uptake and antitumor effect using nanoparticle-based Mcl1-siRNA, followed by nanoparticle-based PTX, PTX, and siRNA, in human epithelial carcinoma KB cells [124]. Trilysinoyl oleylamide-based cationic liposomes were synthesized for the co-delivery of the anticancer drug, suberoylanilide hydroxamic acid (SAHA), and in this case Mcl1-siRNA also showed positive results [125].

The mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) inhibitor blocks the Raf/MEK/extracellular signal-related kinase (ERK) pathway and is involved in proliferation and anti-apoptosis [126, 127]. Kang *et al.* formulated N', N''-dioleylglutamide (DG)-containing liposomes for co-delivery of siMcl1 and the MEK PD0325901 inhibitor, which showed enhanced antitumor activity [128].

Chemosensitivity

Chemo-resistance is a biological response resulting from various signaling pathways, such as inhibition of apoptosis and DNA repair, and is associated with elevated levels of key molecules such as EGFR, VEGF, IGF, Mcl-1 *etc.* Downregulation of relevant signaling molecule expression via a specific gene silencing strategy enhances the chemotherapeutic efficacy in HNSCC [129-132]. Neither chemotherapeutic drugs nor nucleic acids alone can completely eradicate NHSCC, but a combination may prove effective. Synergistic effect mechanisms from nucleic acids not only inhibit certain targeting antigen by complementary sequences, but also guide the drugs into the tumor cells.

Rait *et al.* investigated phosphorothioate pentadecamer ASOs targeted to HER-2 mRNA (antisense HER-2), complexed with a folate-liposome delivery system, to improve the sensitivity to four chemotherapeutic drugs (cisplatin, taxotere, doxorubicin, and 5-fluorouracil) of a low HER-2-expressing and cisplatin-resistant SCC-25CP cell line. Results showed that folate-targeted liposome formation significantly increased intracellular ASOs uptake compared to a lipofectin carrier and free ASOs, accompanied by decreased HER-2 protein levels and increased apoptosis. The combination of a folate-liposome with HER-2 and chemotherapeutic agents showed a synergistic anti-tumor effect, resulting in increased apoptosis. In addition, confocal microscopy revealed that ASOs accumulated mainly in the cell nuclei, while liposomes remained in the cytoplasm after internalization [133]. This study showed that folate-targeted liposome-mediated antisense HER-2 was a potential chemosensitizer in HNSCC, and over-expression of HER-2 was not necessary.

The research using anti-CK2α/α' oligodeoxynucleotides (ODNs) described above, also revealed that knockdown of the CK2 subunits via a specific siRNA, differentially decreased cell proliferation, inhibited cell migration, and enhanced the sensitivity to cisplatin in UM-SCC cell lines. Additionally, CK2α had the greatest effect on modulating proliferation, apoptosis, migration, malignant phenotype and chemosensitivity to cisplatin, while other CK2 subunits showed varying effects on regulation of the cell cycle, migration, and angiogenesis [56].

Fetz *et al.* reported that a combination of survivin-siRNA and an inducible NO synthase (iNOS)-specific inhibitor, 1400W, cooperatively enhanced the chemotherapeutic effects of PTX for HNSCC [134].

5. Radiosensitization

Radiotherapy (RT) is applied widely for primary or adjuvant treatment of HNSCC, with a high tumor control and cure rate, especially in early stage cancer. Despite its benefits, the dose-related treatment toxicity limits the efficacy of RT, which has serious side effects. Moreover, resistance to RT is another problem, which often results in treatment failure. The overall survival rate is only 20% for patients with unresectable tumors treated with radiotherapy alone. The

current clinical strategy for radiosensitization is use of a combination of RT with chemotherapeutics, such as docetaxel (Dtxl), carboplatin; however, this is associated with unacceptable levels of systemic toxicity [135, 136]. Considering the drawbacks of conventional chemo-radiotherapy, targeted nanomedicine represents a promising alternative. (Table 2)

5.1 Glucose transporter-1 (Glut-1)

The exact mechanisms of radioresistance in laryngeal carcinoma remain unclear. Malignant cells frequently encounter a hypoxic microenvironment due to excessive tumor growth. Several studies have shown that GLUT-1 is associated with the malignant glucose metabolism and increased FDG uptake, and predict the hypoxic status of cancer [137-140]. Over-expression of GLUT-1 may be a metabolic marker of radioresistance and an adverse prognosis [141, 142]. This was assessed in laryngeal carcinoma Hep-2 cells, and the results showed a significant difference in GLUT-1 mRNA and protein levels before and after X-ray radiation. Cell survival rates were significantly decreased with increasing doses of radiation and the GLUT-1 ASOs transfection time. In an *in vivo* study, GLUT-1 mRNA and protein levels were reduced after 8-Gy radiation combined with transfection of GLUT-1 ASOs, compared to 8-Gy radiation alone [143]. In conclusion, GLUT-1 ASOs have the potential to act as radiosensitizers for laryngeal carcinoma. It may be possible to use nanotechnology to load GLUT-1 ASOs to enhance gene-silencing efficacy and its radiosensitization effects.

5.2 Folate Receptor

Werner *et al.* reported a polymeric nanoparticle formulation of docetaxel (Dtxl) targeted to the folate receptor (FT-NP Dtxl), and showed higher intracellular uptake by FR-overexpressing KB cells, compared to Dtxl or non-targeted NP Dtxl. Biocompatible and biodegradable poly lactic-co-glycolic acid (PLGA) nanoparticles encapsulated the hydrophobic Dtxl with lipids (lecithin), and lipid-PEG on the surface prevented protein adsorption, with the addition of a surface coating of folate.

The timing of radiotherapy after administration of the radiosensitizer was critical to achieve the maximum effect. In an *in vitro* study, free Dtxl had the greatest sensitization effect when radiation was given initially after drug administration, followed by a slow decrease over time, while the optimal timing of radiotherapy for FT-NP Dtxl was 24 h after drug administration, with the cytotoxicity being as effective as free Dtxl, possibly due to the delayed release of Dtxl from NPs. In an *in vivo* tumor model, FT-NP Dtxl produced the greatest sensitization effect when radiation was applied 12 h after systemic drug administration, and yielded better results than NP Dtxl and free Dtxl [144]. However, there was a rapid increase in tumor volume ~20 days after treatment; this is known as accelerated repopulation, due to cell-death-stimulated increased cell proliferation, which represents a serious problem in terms of tumor recurrence post-therapy [145].

5.3 Epidermal growth factor receptor (EGFR)

Increased expression of EGFR may play an important role in the enhanced cellular proliferation and angiogenic response after exposure to ionizing radiation, leading to RT resistance and/or recurrence [146]. Recent research has indicated significant effects of EGFR inhibitors as chemo- and radiosensitizers. A phase III randomized trial of EGFR inhibitors (CET) as radiosensitizers for locoregionally advanced head-and-neck cancer reported significantly increased survival [147].

Table 2. An outline of targeted nanodevices as radiosensitizers in head and neck cancer therapy

Targeting antigen	Nanoparticles	Radiosensitization	Ref.
folate receptor	Polymeric NPs	FT-NP Dtxl	[144]
EGFR	PLGA NPs	antisense-EGFR NPs	[149]
	Carbon NPs	Cet-targeted PTX-load carbon NPs (noncovalent assembly)	[104]
ATM	PLGA NPs	Antisense-ATM NPs	[150]
Hsp27	lipofectamine	lipofectamine-carried Hsp27 ASOs lipofectamine-carried Hsp27 siRNA	[156] [157]
MnSOD	Liposome	MnSOD-plasmid liposome MnSOD-plasmid liposome+ Gefitinib	[162, 163]
SphK1	Au-NP	SphK1 siRNA -AuNP	[170]

Abbreviations: NPs, nanoparticles; FR, folate receptor; Dtxl, docetaxel; EGFR, Epidermal growth factor receptor; PLGA, poly lactic-co-glycolic acid; Cet, Cetuximab; PTX, paclitaxel; ATM, ataxia-telangiectasia-mutated; MnSOD, manganese superoxide dismutase; SphK1, sphingoid base Sphingosine K1.

Polymeric nanoparticles have also attracted a great deal of interest as potential nanocarriers due to their stability in various environments, the ability to load hydrophobic moieties, controlled drug release, inexpensive and convenient manufacture, and biodegradable properties [18, 148]. The combination of radiotherapy and 24-h–pre-treatment EGFR-inhibition with antisense EGFR polymeric NPs showed a synergistic antitumor effect on the HNSCC SCCVII cell line [149]. PLGA NP-based antisense oligonucleotides targeting the ataxia-telangiectasia-mutated (ATM) gene were also preferentially taken up by SCCVII cells and induced radiosensitization [150].

CET and PTX enhance the radiotherapy sensitivity of HNSCC [151]. Sano *et al.* investigated the noncovalent assembly of CET-targeted PTX-loaded carbon nanoparticles (CET/PTX/PEG-HCCs) as radiosensitizers in OSC-19 and HN5 cells, and found significant inhibition of tumor growth *in vivo* and *in vitro*, compared to other treatment groups [104]. While CET/PTX/PEG-HCCs showed no significant difference compared to the other PTX treatment groups, the combination of CET/PTX/PEG-HCCs and radiation was significantly more effective.

5.4 Heat shock protein 27 (Hsp27)

Hsp27 is a "survival protein" that protects cells from stress stimuli, including heat shock and oxidative stress [152, 153]. High expression of Hsp27 is closely associated with tumorigenesis in various cancers including, breast, prostate, head-and-neck, and colon cancers, and is regarded as a prognostic factor for a poor outcome and therapeutic resistance [154, 155].

Aloy *et al.* demonstrated the protective role of Hsp27 against radiation-induced apoptosis, and presented the associated mechanisms; delayed redox status alteration and mitochondrial dysfunction. The radiosensitization effect of Hsp27 knockdown via Lipofectamine® 2000-carried ASOs or siRNA in SQ20B cell lines was also investigated, and was characterized by over-expression of Hsp27, either constitutively or following irradiation. Both the antisense Hsp27 group and the siRNA-Hsp27 group showed positive results, while the siRNA-Hsp27 groups exhibited a greater decrease in Hsp27 expression and increased cell apoptosis, indicating that siRNA-Hsp27 induced greater radiosensitization [156]. An *in vivo* study confirmed the positive radiosensitive effects of second-generation ASOs targeted to Hsp27, in terms of enhanced radiation-induced SQ20B tumor regression and increased SQ20B survival in mice, without significant toxicity [157].

5.5 Manganese superoxide dismutase (MnSOD)

Radioprotective gene therapy via liposome-delivered MnSOD-plasmids (MnSOD-PL) can protect normal tissues but not tumors, with no adverse effect on the therapeutic response [158, 159]. The mechanism may involve an antioxidant effect and decreased apoptosis [160, 161]. Application of MnSOD-PL gene therapy is at present undergoing clinical trials for the prevention of mucositis during HNSCC combination therapy.

Epperly *et al.* demonstrated the radiosensitive effect of MnSOD-PL in a CAL-33 orthotopic mouse-cheek tumor model, and showed that the EGFR antagonist, gefitinib (Iressa) further enhanced MnSOD-PL transfection-mediated radiosensitization of SCC-VII cells *in vitro* [162, 163].

5.6 Sphingosine kinase 1 (SphK1)

Gold nanoparticles (Au-NPs) have been developed to form radiosensitizers [164]. Hainfeld *et al.* tested Au-NPs as radiosensitizers in a SCCVII model and showed a reduced tumor control dose 50% (TCD50) and increased long-term survival. The data also indicated the effects of the radiation dose, beam energy and hyperthermia [165].

Au-NPs can serve as efficient carriers for many therapeutic agents, due to their convenient surface-modification, biocompatibility, enhanced cellular internalization, controlled payload release and non-toxicity [166]. In addition, the phenomenon of surface plasmon resonance (SPR) facilitates label-free monitoring of the distribution of these complexes [167].

Acylated sphingoid base ceramide (Cer) and sphingoid base sphingosine (Sph) are known tumor suppressor lipids that inhibit proliferation and promote apoptosis, while sphingosine-1-phosphate (S1P) exhibits the opposite effects. Sphingosine kinases (SphKs), such as SphK1, can phosphorylate Sph to form S1P, which contributes to maintenance of a dynamic equilibrium between cell proliferation and death [168]. Reduced levels of Cer and Sph, accompanied with increased levels of SphK1 and S1P, are implicated in various human cancers and are associated with anti-apoptotic phenotypes, tumor aggression and radioresistance; they are therefore regarded as new targets for cancer therapies [169]. Free-form siRNA molecules have a very short circulation time in the physiological environment due to their vulnerability to degradation and clearance. Masood *et al.* used gold nanorod (GNR) technology to deliver SphK siRNA into HNSCC cells, and found enhanced gene silencing efficiency and significant radiosensitization *in vitro* and *in vivo*, at a dosage 5 × lower than that

used commonly in pre-clinical RT. This experiment confirmed the radiosensitizing effects of Au-NPs, which were enhanced in combination with a SphK siRNA gene silencing strategy. Post-mortem analysis showed increased intracellular GNR-siRNA nano-plexes compared to GNR alone, while GNR was not detected in adjacent normal tissue, demonstrating its exclusive uptake by tumor tissues [170].

6. Photothermal therapy (PTT)

PTT is a localized, minimally invasive treatment using near-infrared (NIR). The concentrated light activation of a photosensitizer (PS), which accumulates preferentially in tumor tissues, induces a toxic photodynamic reaction to generate reactive oxygen species and active a cascading series of events, finally leading to oxidative damage and cell death [171].

A major advantage of PDT over chemo- or radiotherapy is that both PS and the irradiating light are themselves essentially inert and hence cause minimal systemic toxicity, which enables PDT to be used for repeated treatments of patients with HNSCC [172]. However, photo-toxicity arising from the unintentional systemic distribution of the PS and the efficacy of the treatment remain considerable problems in PDT. Foscan® is an ethanol/propylene glycol formulation of the PS temoporfin, and has been approved in Europe for the treatment of advanced and recurrent HNSCC. However, it failed to achieve FDA approval in the U.S. for the treatment of HNSCC due to poor tumor-selectivity and serious cutaneous burns [173]. Improvement of precise tumor-targeting is needed for a clinical application of PDT.

Schneider *et al.* reported two conjugates of FR-targeted tetraphenylporphyrin (TTP) as selective photosensitizers that displayed at least sevenfold greater uptake by KB nasopharyngeal cells compared to a TTP control [174]. Another selective photothermal therapy using anti-EGFR conjugated Au-NPs had been shown to be efficient in two oral squamous carcinoma cell lines (HSC 313 and HOC 3 Clone 8) [175]. More recently, the photosensitizer, silicon phthalocyanine-4 (Pc 4), was packaged in a polymeric micelles nanoplatform surfaced with GE11 peptides targeted to EGFR. The resulting EGFR-targeted Pc 4-nanoformulation was preferentially taken up by EGFR-positive H&N SCC-15 cells and showed a significant anti-tumor effect *in vitro* and *in vivo* [176].

7. Future perspectives

Nanoparticle-based targeted therapeutics is a hot topic in cancer therapy. Though most approaches are still in the preclinical stages, they have shown tremendous potential to fulfill the need for viable alternative cancer therapies. Further research into high-er-specificity tumor targets and more efficient nano-carriers is needed, including complex modifications to enhance the antitumor efficacy, and to achieve the ultimate goal of personalized medicine.

Competing Interests

The authors have declared that no competing interest exists.

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